EXHIBIT 1

1 OF 3

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EXHIBIT 1

University of Cincinnati
Medical Center

College of Medicine
John E. Maggio, Ph.D.
Department of Pharmacology and Cell Biophysics
University of Cincinnati
PO Box 670505
Cincinnati, OH 45237-0505
2120 East Galbraith Road
Room 237, Building A
Cincinnati, OH 45237-0505
Phone: (513) 558-2354
Email: John.Maggio@uc.edu

1 August 2011

Janet Abaray, Esq.
Burg, Simpson, Eldredge, Hersh, Jardine, PC
312 Walnut Street, Suite 2090
Cincinnati, OH 45202

Re: In Re: Yaz/Yasmin/Ocella Product Liability Litigation, MDL No. 2100, United States District Court, Southern District of Illinois

Dear Ms. Abaray:

I. Introduction

1. This is a report on the pharmacology of hormonal contraception, particularly as related to the steroid drugs ethinyl estradiol and drospirenone, as delivered in combination oral hormonal contraceptives. Among the contraceptive products containing ethinyl estradiol and drospirenone presently sold are *Yasmin®*, *Ocella®*, *Syeda®*, *Zarah®*, *Beyaz®*, *Safyral®*, *Yaz®*, *Yasminelle®*, *Gianvi®*, and *Loryna®*. Since *Yasmin®* (a Bayer product first approved by FDA in May 2001) was the first of these products launched, the product group has been called the "*Yasmin®* family" of oral contraceptives. I will also discuss such related topics as the pharmacokinetics, mechanisms of action, safety and efficacy of hormonal contraceptives. This report reflects my expert opinion, to a reasonable degree of scientific certainty, based on the information I have reviewed to date; I must reserve the right to review or update this document based on additional information. In forming the opinions expressed herein, I have used the same analytical and decision-making processes that I customarily employ when rendering scientific opinions in my profession as a pharmacologist. I address here aspects of the general pharmacology of the estrogens and progestins used in hormonal contraceptives, as well as pharmacology specifically related to drospirenone-containing hormonal contraceptives. I have tried here to minimize facts that are well known and easily available elsewhere. I have attempted to keep the text simple in the interest of wider comprehensibility, but will discuss these matters at a more precise and technical level if requested. At the end of this report are three appendices. Appendix I lists the abbreviations used in the report; Appendix II discusses materials available for my review; Appendix III is a timetable of selected events.

II. Information Considered

2. Much of the material I have reviewed in preparing this report consists of publicly available medical and scientific literature, including primary publications, review articles, monographs and textbooks. Among the other sources I have reviewed are ones publicly available online, notably at the websites of the US Food and Drug Administration and the National Library of Medicine. I have relied to a limited extent on materials I have written myself for the purpose of educating physicians. I have also read some public information written for the layman rather than for the expert. All these sources are referenced below within the text. I have also reviewed documents from the In Re: Yaz/Yasmin/Ocella Product Liability Litigation, MDL No. 2100, United States District Court, Southern District of Illinois multi-district litigation; these are referenced below by "BHCPYAZ" or "BSPYAZ" plus a multi-digit number identifying the document and page. A complete list of materials available for my review accompanies this report as a separate document.

3. I have particularly consulted the following textbooks, commonly used in the training of physicians, for information about the pharmacology of hormonal contraceptives.

Goodman and Gilman's The Pharmacological Basis of Therapeutics, 11th Edition, L. L. Brunton *et al.*, Eds., McGraw-Hill, 2006.

Basic and Clinical Pharmacology, 10th Edition, B. G. Katzung, Ed., Lange, 2007.

Basic and Clinical Pharmacology, 11th Edition, B. G. Katzung, Ed., Lange, 2009.

McGraw-Hill's AccessMedicine (an online textbook), 2007ff. http://www.accessmedicine.com/popup.aspx?aID=953748.

Principles of Pharmacology, D. E. Golan *et al.*, Eds., Lippincott Williams & Wilkins, 2005.

Principles of Pharmacology, 2nd Edition, D. E. Golan *et al.*, Eds., Lippincott Williams & Wilkins, 2008.

Principles of Pharmacology, 3rd Edition, D. E. Golan *et al.*, Eds., Lippincott Williams & Wilkins, 2012.

III. Background and Qualifications

4. I am a pharmacologist, and have pursued this career for more than twenty-five years. I hold an endowed chair as the Flor van Maanen Professor of Pharmacology and Experimental Therapeutics (1997-date) and for ten years (1997-2007) was Director (Chairman) of the Department of Pharmacology and Cell Biophysics at the University of Cincinnati College of Medicine (Cincinnati, OH). I also hold an academic appointment at Harvard Medical School (Boston, MA), where I am Instructor of Neurology. I am a research scientist with many publications and have held many peer-reviewed grants and sat on many scientific review committees.

5. For about ten years, I directed the education of medical students in pharmacology at my institution, and as such, was the person most directly responsible for the preparation of medical students for licensure, clinical training, and practice in the area of pharmacology. The course of study I directed (given largely to second year medical students and Ph.D. students) included

several lectures and accompanying written syllabus chapters and slidesets prepared by myself. Among these are several topics in general pharmacology (introduction to pharmacology, origin of drugs, history of regulation, clinical trials, pharmacodynamics, absorption, distribution, metabolism and elimination of drugs, pharmacokinetics, and drug delivery) upon which I consider myself to be an expert. Also among the fields in which I consider myself a particular expert are kinetics, radioimmunoassay, chromatography, and chromatography coupled with mass spectrometry, and many of my original publications treat one or more of these topics. These are fields especially relevant to the matters I discuss below. I also train future physicians and scientists in central nervous system pharmacology (*e.g.*, dopamine pharmacology, Parkinson's disease, antipsychotics, Alzheimer's disease and antidepressants) and in safety pharmacology.

6. Pharmacology, in laymen's terms, is the science of how drugs interact with the body and how the body interacts with drugs. The former broad area is called pharmacodynamics, and the latter broad area is called pharmacokinetics. The discovery of drugs, the study of their therapeutic effects and adverse effects, and the experimental use of drugs to understand biological systems are all parts of pharmacology. [Pharmacology is very different from pharmacy, which is the practice of dispensing drugs.]

7. I have served on the faculty of Harvard Medical School (1985-1997 and 2007-date) and University of Cincinnati College of Medicine (1997-date). My complete *Curriculum Vitae* accompanies this report as a separate document. Medical education and research is my career, and I train physicians and am consulted by them. However, my formal training does not include an M.D. degree or a license to practice clinical medicine. Thus I do not consider myself an expert in the clinical practice of medicine.

IV. Hormonal Cycles and Fertility

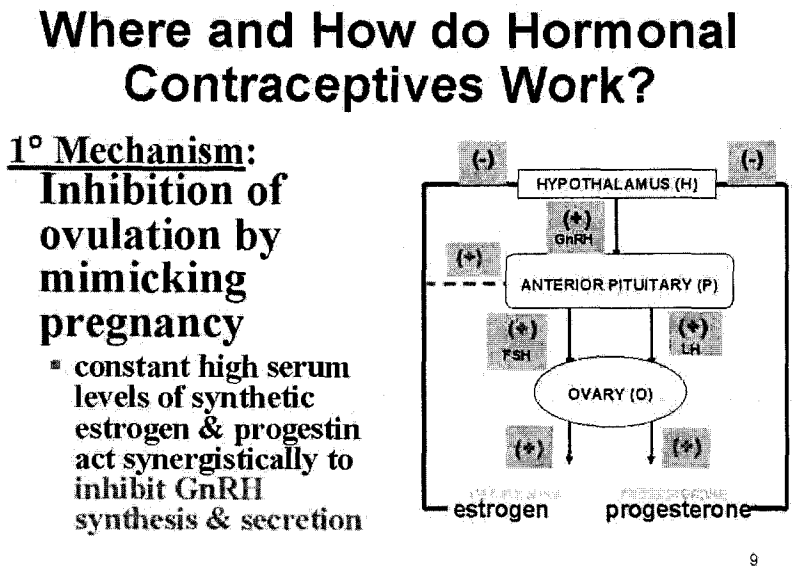
8. The hormonal systems that control menstrual cycles, fertility and pregnancy in female mammals are quite intricate. There are several hormones, both peptide and steroid in nature, whose levels rise and fall (*i.e.*, cycle) in distinct patterns with time. Among the key steroid hormones are the natural estrogens and progestins. These hormones affect, and are affected by, cycles in other hormones through complex "feedback loops". Normal fertility in female mammals requires synchronization of several biochemical pathways. The hypothalamus and pituitary in the brain and the ovary in the abdomen (together, the "HPO axis") are all essential to fertility and communicate with each other via hormones.

9. "Estrogens", "progestins" and "androgens" are names of classes of steroid molecules which will be discussed in more detail below. In broad terms, estrogens are feminizing and androgens are masculinizing. Progestins exert antiproliferative effects on the female endometrium (thus promoting secretion over the proliferation induced by estrogens) and are required for maintenance of pregnancy. [Progestins are sometimes known by the older term progestogens.] All these hormones are biosynthetically derived from cholesterol.

10. Two important hormones secreted by the ovary are the steroids 17β-estradiol (the major naturally-occurring estrogen) and progesterone (the major naturally-occurring progestin). Among other activities, these hormones act on the hypothalamus (part of the brain) to inhibit release of gonadotropin releasing hormone (GnRH), an inhibition which ultimately (through

several steps) leads to inhibition of ovulation. Inhibition of ovulation during pregnancy (a phenomenon important for successful reproduction; *vide infra*) is mediated by a rise in the levels of the natural hormones 17β-estradiol and progesterone during pregnancy and the maintenance of those high levels (rather than monthly cycling) during pregnancy. In the premenopausal nonpregnant mature female, most of the circulating estrogen comes from the ovary; in the pregnant female, most of the circulating estrogen is produced by the fetoplacental unit (Goodman & Gilman; Katzung; *op. cit.*).

11. The figure below shows the feedback loops, and how estrogens and progestins inhibit GnRH release, and ultimately inhibit ovulation. Hormonal contraceptives act on and through these pathways.



12. This inhibition-of-ovulation activity of estrogens and progestins is essential for understanding the mechanism of action of hormonal contraceptives. Hormonal contraceptives are sometimes said to act by "mimicking pregnancy". What this means is that hormonal contraceptives, by delivering higher and more constant levels of estrogens and progestins than present in normal menstrual cycles, inhibit ovulation in the same way as the higher levels of estrogen and progestin during pregnancy inhibit ovulation, specifically by inhibiting secretion of GnRH from the hypothalamus. That inhibition of ovulation is the primary mechanism by which oral contraceptives block fertility. Secondary mechanisms include impairment of blastocyst transport and implantation, increasing the viscosity of cervical mucus, and inhibition of fallopian tube motility, as shown in the figure below. These mechanisms account for the very high efficacy (>>99% in fully compliant or "perfect" use) of combination oral contraceptives (Golan, 2008, *op. cit.*).

13.

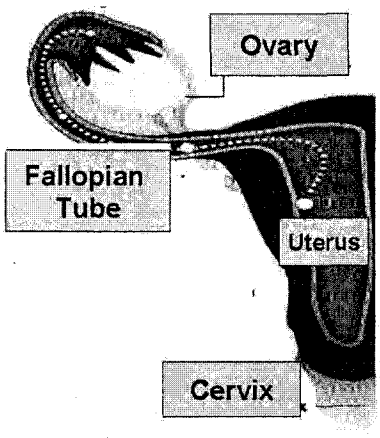
How Do Hormonal Contraceptives Work?

1° Mechanism:

Inhibit ovulation, by "mimicking pregnancy"

2° Mechanisms:

Impair fertilization, blastocyst transport & implantation.



V. Historical Introduction to Hormonal Contraception

14. It was recognized centuries ago that the reproduction of humans and other placental mammals would be imperiled if a second pregnancy were to begin while a first pregnancy was incubating. Consider, for example, two human fetuses differing in age by four months sharing the same uterus at the same time. Such a situation would almost surely put both fetuses and the mother at grave risk. But such a dangerous situation, which would surely imperil reproduction, is virtually impossible because there are powerful biological mechanisms to prevent its occurrence. Specifically, conception is efficiently prevented during an established pregnancy by suppression of ovulation.

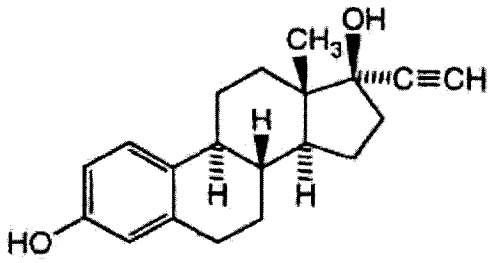
15. The observations above led to the concept that ovulation is suppressed during pregnancy by one or more hormones secreted during pregnancy. [A hormone is a molecule that is released by one cell or tissue, carried by the bloodstream, and has a physiological effect on a distant cell or tissue.] This hypothesis was well known a century ago, and pharmacologists of that time speculated that a preparation of the hormone(s) that suppress ovulation during pregnancy could prevent ovulation (and thus prevent conception) in a nonpregnant sexually active mature female placental mammal. Proof-of-principle of oral hormonal contraception was established as early as 1927 by the experiments of Haberlandt, which demonstrated that feeding ovary and placenta extracts to healthy female rats rendered them temporarily infertile. Serious research toward development of oral contraceptives accelerated over the next decades.

16. By the 1950s, naturally-occurring steroid hormones (estrogens and progestins) involved in human fertility had been identified, and it was known that some of these could prevent ovulation in nonpregnant women. For both pharmacokinetic reasons (poor bioavailability and

rapid metabolism; *vide infra*) and economic reasons (the natural hormones are expensive to produce in the required quantities), the native molecules are impractical as oral contraceptives. However, close chemical cousins of the native hormones (with higher bioavailability and slower metabolism) are practical as contraceptives. Thus synthetic non-native estrogens (*e.g.*, mestranol or ethinyl estradiol) and progestins (*e.g.*, norethynodrel or norgestimate), rather than the native hormones, were and are used in hormonal contraceptives. The first clinical trials of oral contraceptives were completed in the 1950s. The trials demonstrated high efficacy, and in 1960 the first oral contraceptive ("OC"), or birth control "Pill", was approved by FDA.

17. The chemical structures of the estrogens and progestins considered here are well known. The major native estrogen is 17β-estradiol, with hydroxyl (-OH) groups at the 3 (ring A) and 17 (ring D) positions of the steroid nucleus. Ethinyl estradiol ("EE") is identical to 17β-estradiol but for the addition of an acetylenic ethinyl group at position 17 of ring D, a modification which makes EE more orally potent and long-lived than estradiol, largely because EE is less vulnerable than estradiol to first-pass metabolism by the hepatic P_{450} enzyme CYP-3A4 (Loose & Stancel, *Estrogens and Progestins* (Chapter 57), in Goodman & Gilman, 2006, *op. cit.*). Mestranol is the 3-O-methylether derivative of ethinyl estradiol. Mestranol is actually a "pro-drug"; it is rapidly demethylated in the body to ethinyl estradiol, its active form. Thus mestranol and ethinyl estradiol have very similar potency and pharmacokinetics (Goodman & Gilman, 2006; Katzung, 2007; *op. cit.*). Ethinyl estradiol is the estrogen used in the *Yasmin*® family of contraceptives and in most oral contraceptives. The structure of EE is shown in the figure below.

18. Structure of ethinyl estradiol. Mestranol differs by having a methoxyl (-OCH_3) rather than a hydroxyl (-OH) group at position 3 of ring A, in the lower left corner of the schematic.

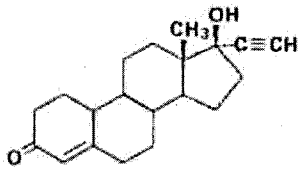


Ethinyl Estradiol

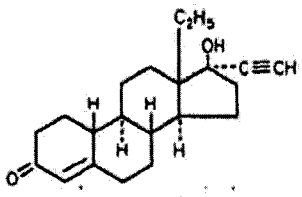
19. The major native progestin is progesterone, and the contraceptive progestins to be discussed here are derivatives of the progesterone nucleus. The structures of some of these progestins are shown in the figure below. The pharmacology of progestins used in hormonal contraceptives, and the progestin used in the *Yasmin*® family of oral contraceptives, drospirenone, are discussed in detail later.

20.

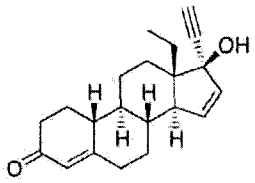
Progestins used in COCs



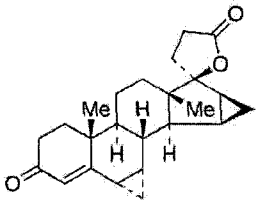
Norethindrone (1st gen)



Levonorgestrel (2nd gen)



Gestodene (3rd gen)



Drospirenone (4th gen)

21. The progestins used in oral contraceptives have been divided into "generations" on the basis of their dates of introduction, their various pharmacological activities, and/or their chemical structures. These multiple classification schemes can lead to confusion, as the same progestin might be classified in different generations depending on the scheme employed in a particular reference. [Unfortunately, this is not an unusual situation in pharmacology.] I have chosen to in this report to classify contraceptive progestins based on their pharmacological activities, the classification I believe is in most common use and most relevant to the topics I will discuss. I recognize that others may use structural or historical or metabolic bases for defining generations of progestins. I discuss generations of progestins further below.

22. The first OC approved in the US was *Enovid*®. It contained 150 μg/day estrogen (the particular estrogen used was mestranol) and 9850 μg progestin (the particular progestin used was norethynodrel). The very high doses of synthetic hormones used in the early ("first-generation") OCs soon proved unsafe and unnecessary for efficacy, and later OCs had lower doses of estrogen and progestin. *Enovid*® was introduced in 1960, and the first report of a blood clot and pulmonary embolism in a patient using *Enovid*® was published in 1961 (Jordan & Anand, *Lancet* 278: 1146-1147, 1961). Oral contraceptives such as *Enovid*®, which contain both an estrogen and a progestin, are sometimes called combination oral contraceptives ("COCs"). [In the *Yasmin*® family of combination oral contraceptives, the estrogen is ethinyl estradiol ("EE") and the progestin is drospirenone ("DRSP"); chemical structures above.]

23. By the mid-1960s, numerous OCs were available, and these products were used by millions of women. With good patient compliance, they were extremely efficacious as contraceptives (99.8+% over a year with perfect use). With wider use of hormonal contraceptives came more reports of adverse effects. By the late 1960s, epidemiology established an increased risk for venous thrombosis (clot) in OC users and an increased risk of

myocardial infarction (heart attack) in OC users who smoked. These risks of OCs were widely publicized in print (*e.g.*, Seaman, *The Doctors' Case Against the Pill*, 1969) and other media, leading to the mandatory inclusion of side effects and risks on product labels (*e.g.*, *Fed. Regist.* 35: 9001-9003, 1970).

24. In 1969, the United Kingdom (UK) Committee on Safety of Drugs addressed British physicians warning of the higher risk of adverse vascular events in users of oral contraceptives containing higher doses of estrogens. "Reports of suspected adverse reactions received by the Committee on Safety of Drugs now provide evidence that the incidence of thromboembolism is higher among women taking preparations containing larger doses of oestrogen than among those taking preparations containing the smaller dose. … The relation between the use of oral contraceptives and thromboembolism has been well established, but the Committee is of the opinion that the risk of this complication is less with the lower oestrogen dose." In 1970, Inman *et al.* (*Brit. Med. J.* 2: 203-209, 1970) concluded in a peer-reviewed publication that "the data collected independently in the three countries [Sweden, Denmark, and the UK] leave no doubt that there is a positive correlation between the risk of thromboembolism and the dose of oestrogen in oral contraceptives."

25. It was generally recognized by physicians around this time (about forty years ago) that such side effects of OCs were dose-dependent, but this finding was hardly a surprise. Dose-dependency of therapeutic and adverse effects was and is a very well-established principle of pharmacology. Since adverse effects were dose-dependent upon estrogen dose, and since estrogen content of the first-generation OCs was higher than necessary for contraceptive efficacy, OCs that delivered lower doses of estrogens and progestins were developed in the 1970s. The lower dose OCs are sometimes called "second-generation OCs". [Generations of OCs are not the same as generations of progestins used in OCs.] Doses of estrogens and progestins in COCs continued to fall after the 1970s. The 1980s brought phasic products and further reductions in estrogen doses. [Phasic products deliver different doses of hormones during different phases of the monthly cycle. Today monophasic, biphasic and triphasic COCs are available.] Estrogen doses have fallen a great deal from the first OCs (typically ≥100 μg/d) to those used today (typically 10-35 μg/d).

26. By the mid-1980s (more than 25 years ago), it was very well-accepted that higher estrogen doses mean higher risks of various adverse effects, but are not necessary for efficacy. In 1988, this recognition was formalized in a regulatory sense in the US. That year an FDA advisory committee concluded, several years after the conclusion had been reached in Britain, that higher estrogen doses convey higher risks of adverse effects, but were no more efficacious at contraception than lower estrogen doses. FDA forced "high-estrogen" OCs, defined in 1988 as those containing more than 50 μg/day estrogen, off the market that same year. All such OCs, seven products containing as much as 100 μg/day estrogen, were withdrawn later in 1988, after their manufacturers distributed "Dear Doctor" letters. I am not aware of any oral contraceptive containing more than 50 μg/d estrogen being sold in the US after 1988. I am not aware of any hormonal contraceptive product delivering the equivalent of more than 50 μg/day estrogen in an oral contraceptive being sold in the US after 1988, until the *Ortho Evra*® contraceptive patch appeared more than a decade later (approved 2001), and the label of that product carries a black box warning about its higher estrogen delivery.

27. When pharmaceutical products are withdrawn, the manufacturer typically send out a letter of explanation to health care professionals. I have reviewed a "Dear Doctor" letter (30 March 1988) from Ortho Pharmaceutical Corporation withdrawing their OC products containing 80 and 100 μg/day estrogen. That letter included the recommendation that patients "should be continually monitored and moved to lower dose products whenever possible." The latter statement recognizes a well known general principle of medicine, pharmacology and clinical therapeutics: regardless of the particular drug(s) selected, treatment should use the minimum dose and duration needed to achieve the desired therapeutic endpoint. This principle applies to estrogens and progestins as it does to other drugs (*vide infra*).

28. For example, the warning that vascular risks from hormonal contraceptives are dose-dependent appears on the labels of oral contraceptives sold in the United States. "A positive association has been observed between the amount of estrogen and progestogen in oral contraceptives and the risk of vascular disease. … Minimizing exposure to estrogen and progestogen is in keeping with good principles of therapeutics. For any particular estrogen/progestogen combination, the dosage regimen prescribed should be the one which contains the least amount of estrogen and progestogen that is compatible with a low failure rate and the needs of the individual patient." New users have a special warning: "New acceptors of oral contraceptives should be started on preparations containing the lowest estrogen dose which provides satisfactory results in the individual." The particular quotations above comes from the current label of *Yasmin*®, where the "use lowest effective dose" warning appears at least three times, but similar warnings are found on all OC labels.

29. Some OC labels are more explicit about new users: "New acceptors of oral contraceptive agents should be started on preparations containing 0.035 mg (=35 μg) or less of estrogen. Products containing 50 mcg (=50 μg) estrogen should be used only when medically indicated." The latter is quoted from the label of *Ortho-Novum*® *1/50.*

30. From the studies I describe below, it is clear that the *Yasmin*® oral contraceptive delivers more estrogen than its label suggests. In fact, a significant fraction of the patients who use this product are subjected to levels of ethinyl estradiol higher than the mean levels that result from use of 50 μg/d EE product. Thus *Yasmin*® should especially not be prescribed for a new user of hormonal contraceptives. In my opinion, any hormonal contraceptive product, including *Yasmin*®, that delivers more ethinyl estradiol to the bloodstream than a 50 μg/day COC (at steady-state; *vide infra*) to a significant fraction of patients, is inconsistent with the 1988 ban discussed above, and should be clearly labeled as such.

31. The 1990s brought combination OCs containing a new class of progestins, called "third-generation" progestins, distinguished from other progestins by having lower androgenic activity. [Third-generation progestins are discussed in more detail below.] For example, the COC product *Mircette*® (approved 1998) contains 20 μg/day estrogen (EE), vs. 150 μg/day estrogen (mestranol) in *Enovid*®, approved (1960). The third-generation progestin in *Mircette*® is desogestrel (150 μg/d), vs. 9850 μg/day of the first-generation progestin norethynodrel in *Enovid*®. [*Mircette*® information from the 2006 product label, available, as are most or all of the labels of products I discuss in this report, at http://www.fda.gov).] That is, the modern product contains six-fold lower estrogen dosage than the first OC. In 2011, not only pills, but also patches, injections, implants, rings, IUDs, etc., are available for hormonal contraception.

Most COCs available in 2011 contain 10 to 35 μg estrogen (ethinyl estradiol) per daily dose; the progestin and progestin dose vary.

VI. Estrogens as Drugs

32. The major general principles of pharmacology apply to all drugs, and it is important to consider estrogens (and progestins) in this context. Like other drugs, estrogens have therapeutic effects, adverse effects, and contraindications.

33. As with other drugs, the therapeutic effects and adverse effects of estrogens are dose-dependent. A higher incidence of the adverse effects (whether mild, moderate, or severe) of estrogens is expected at higher doses of estrogen. More adverse effects of a drug at higher doses of that drug is a very well-established principle of pharmacology, and certainly applies to estrogens. Paracelsus (1493-1541) noted centuries ago that "the right dose differentiates a poison from a remedy."

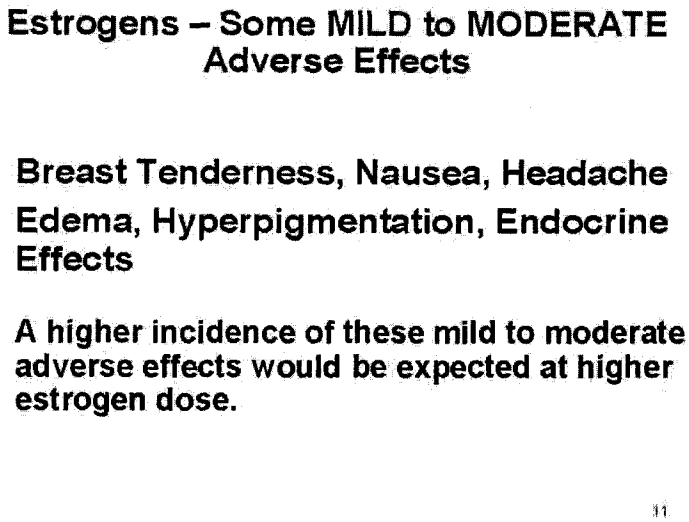
34. Like other drugs, estrogens are risky in overdose. As in treatment with other drugs, treatment with estrogens should use the minimum dose and duration for the desired therapeutic endpoint.

35. Like other drugs, estrogens are absorbed and cleared with timecourses that depend on formulation, delivery, metabolism, etc. These latter processes are subjects of the branch of pharmacology known as pharmacokinetics; *vide infra.*

VII. Adverse Effects of Estrogens

36. Among the mild to moderate adverse effects of estrogens are breast tenderness, nausea, headache, edema, hyperpigmentation, and endocrine effects. A higher incidence and greater severity of these mild to moderate adverse effects would be expected and is observed at higher estrogen doses. Such mild to moderate adverse effects are not rare in patients using oral contraceptives; about a third of patients who start using hormonal contraceptives discontinue use for reasons other than to become pregnant (Chrousos, *The Gonadal Hormones and Inhibitors* (Chapter 40) in Katzung, 2007, *op. cit.*). These mild to moderate adverse effects of estrogens as drugs are predictable from the biological activities of estrogens. For example, estrogens are largely responsible for the growth of breast tissue (and other female secondary sexual characteristics) at puberty, and for the regional pigmentation of nipples and areolae during pregnancy; thus it is not surprising that breast tenderness and hyperpigmentation are adverse effects of estrogens as drugs. [Blood levels of natural estrogens increase during puberty and increase more during pregnancy.]

37. Some of the mild adverse effects of estrogens are summarized in the figure below.



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38. Prominent among the serious adverse effects of estrogens are cardiovascular problems such as venous thromboembolism (clots), myocardial infarction (heart attack) and cerebrovascular disorders (*e.g.*, stroke). These are further discussed below. Again, the serious cardiovascular adverse effects of estrogens are consistent with the biological activities of these drugs. For example, estrogens enhance coagulability of blood (predisposing to clots), show vasomotor activity (*e.g.*, hot flashes at menopause are vasomotor symptoms, and are treated with low dose estrogen, much lower doses than in hormonal contraceptives). The risk for many of these cardiovascular problems is particularly marked in women over 35 years of age who smoke heavily (defined as >15 cigarettes/day) during use of OCs (Goodman & Gilman, 2006; Katzung, 2007; *op. cit.*). The latter is a blackboxed warning on the labels of oral contraceptives.

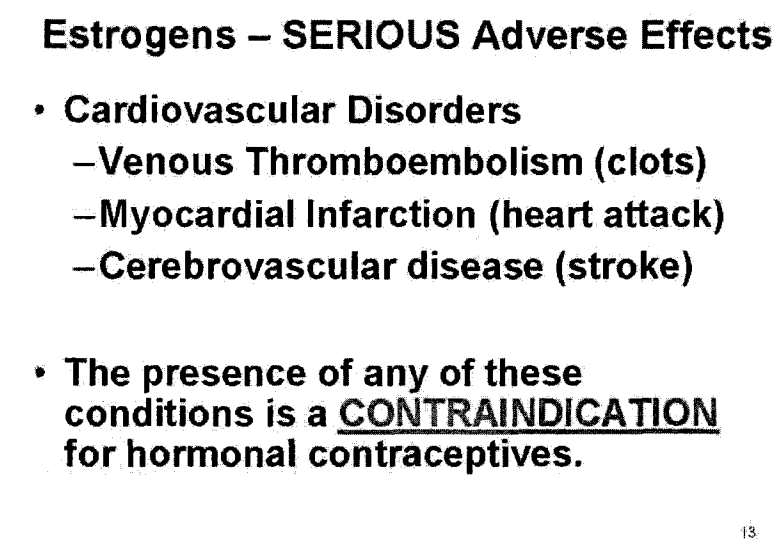
39. The higher risk for smokers vs. nonsmokers does not apply to venous thromboembolism ("VTE"). VTE risk is increased in the first month of use of estrogen-containing OCs, remains high over years of use, and returns to normal by a month after discontinuing use. This temporal relationship clearly points to estrogens as a one cause of VTEs (Stadel, *NEJM* 305: 612-618, 1981; Goodman & Gilman, 2006; Katzung, 2007; *op. cit.*).

40. The presence of any of the above cardiovascular disorders constitutes a contraindication for hormonal contraceptives (Goodman & Gilman, 2006; *op. cit.*). Similarly, all of the following are considered contraindications for combination oral contraceptives: coronary artery disease; carcinoma of breast or uterus, or other hormone-responsive neoplasias; abnormal vaginal bleeding; pregnancy or impaired hepatic function.

41. The relative risk to users of OCs for various adverse effects can be found in any COC product label. Some examples from the *Yasmin*® label follow. Myocardial infarction, 2-6 fold; superficial vein thrombosis (first episode), 3-fold; deep vein thrombosis or pulmonary embolism, 4-11 fold; post-operative thrombosis, 2-4 fold; thrombotic strokes, 3-14 fold.

42. Relative contraindications for estrogen-containing OCs are also in line with the known pharmacology and adverse effects of estrogens. These include migraine headache (headache is a mild to moderate adverse effect of estrogens; *vide supra*) and hypertension (cardiovascular effects; the early "first-generation" (high dose estrogen) OCs caused hypertension in 4-5% of normotensive patients and increased blood pressure in 10-15% of those with pre-existing hypertension).

43. Some of the serious adverse effects of estrogens are summarized in the figure below.



44. The enhanced risk of venous thromboembolism (VTE) caused by COCs is mediated by effects of estrogens and progestins on the liver, where they can affect the levels of proteins involved directly or indirectly in clotting, thus producing a prothrombotic (hypercoagulable) state. Changes in pro-coagulant, anti-coagulant and fibrinolytic pathways and proteins all occur (Battaglioli & Martinelli, *Curr. Opin. Hematol.* 14: 488-493, 2007; Tchaikovski & Rosing, *Thromb. Res.* 126: 5-11, 2010). A prominent effect of COCs on the anticoagulant pathway is induction of acquired resistance to activated protein C (APC). Resistance to APC (APC resistance or APCres) is an independent risk factor for VTE (de Visser *et al.*, *Thromb. Hemost.* 93: 1271-1276, 1999). APCres is higher with third-generation than second-generation progestins (Battaglioli & Martinelli, *op. cit.*; Tans *et al.*, *Thromb. Hemost.* 84: 15-21, 2000). That would be consistent with the observation that users of COCs containing third-generation progestins are at greater risk for VTEs than users of COCs containing second-generation progestins (epidemiology discussed in Sections XII and XIII in this report).

45. Essentially all clinical studies of COCs consider the effects of the medications on various serum proteins synthesized by the liver. One of these is sex hormone binding globulin, or SHBG. The level of this protein increase markedly after a patient begins taking a COC containing estrogen, and this increase in SHBG levels (other things being equal) is related to the dose of ethinyl estradiol. Some consider the rise in SHBG a measure of the net "estrogenicity" of a COC regimen (*vide infra*). The progestin component also contributes to SHBG levels, with some progestins showing a distinct antagonistic effect on the SHBG

induction and other activities of EE in COCs (Kuhl, *Drugs* 5: 188-215, 1996; Kauppinen-Mäkelin *et al.*, *Clin. Endocrinol.* 36: 203-209, 1992; Jung-Hoffmann *et al.*, *Contraception* 38: 593-603, 1988).

VIII. Progestins

46. The same principles of pharmacology discussed above (Section VI) apply to progestins as well as to estrogens. Progestins have therapeutic effects and adverse effects which are dose-dependent, and there are contraindications for their use. Like estrogens, progestins are absorbed and cleared with timecourses that depend on formulation, delivery, metabolism, etc. (pharmacokinetics). Like estrogens, progestins display a variety of biological activities that must be considered when discussing their pharmacology.

47. Whereas nearly all COCs use the same estrogen (ethinyl estradiol, or EE), there are a large number of progestins in common use. These progestins differ in their pharmacology, making discussion of their biological activities somewhat more complex than that of estrogens.

48. Progestins used in COCs are classified into first, second, third, etc., "generations" on the basis of their pharmacological activities, as shown in the chart below. I have omitted some pharmacological activities and several progestins in the interest of simplicity. For example, the chart gives only one example compound of each progestin generation.

49.

Generations of Progestins

Activity:	PR	ES	AND	AA	GLU	AM
1st Generation (*e.g.*, norethindrone)	++	++	++	-	-	-
2nd Generation (*e.g.*, levonorgestrel)	+++	-	+++	-	-	-
3rd Generation (*e.g.*, desogestrel)	++	-	+	-	-	-
4th Generation (*e.g.*, drospirenone)	+	-	-	+	-	++

PR=progestational, ES=estrogenic, AND=androgenic,
AA=antiandrogenic, GLU=glucocorticoid, AM=antimineralocorticoid

Note that drospirenone, the progestin in the *Yasmin*® family of COCs, is distinctly different in its pharmacodynamics from earlier generations of progestins. In particular (**red**), DRSP lacks the androgenicity shown by earlier compounds, and shows antiandrogenic and antimineralocorticoid activities that earlier compounds lack. See text for further explanation.

50. Theoretically, an ideal synthetic progestin would be active only at progesterone receptors (Golan, 2008, *op. cit.*), but early synthetic progestins showed also activities on other systems, and were both estrogenic and androgenic. For example, first-generation progestins (*e.g.*, norethindrone, norethynodrel) showed an adverse effect on lipid metabolism (Hirvonen *et al.*, *New Engl. J. Med.* 304: 560-563. 1981; Benagiano *et al.*, *Europ. J. Contracep. Reproduct. Health* 9: 182-193, 2004), leading to an effort by the pharmaceutical industry to synthesize progestins with metabolic neutrality (avoiding lipid effects) and higher potency (allowing lower doses).

51. The result of that effort was the second-generation compounds (*e.g.*, levonorgestrel), a novel class of steroids which are still used in COCs. The effort was successful; *e.g.*, norgestimate is more potent than norethindrone at the progesterone receptor, but about 50 times less potent at estrogen receptors (Benagiano, 2004, *op. cit.*). First- and second-generation progestins showed varying degrees of androgenic activity, leading to symptoms (*e.g.*, acne, seborrhea, hirsutism) which were recognized as side effects of these compounds.

52. A third generation of progestins with lower androgenic activity than second-generation drugs was later developed, and lower androgenicity is a key characteristic of the third-generation progestins as a class. Examples of third-generation progestins include desogestrel, gestodene and norgestimate; all the latter are derived structurally from the second-generation progestin levonorgestrel (Benagiano, 2004, *op. cit.*). [Some authors consider norgestimate a second-generation progestin based on the time of its appearance, or on the basis that it is metabolized by the liver to the second-generation compound levonorgestrel.] In some ways, the androgenic activity of progestins is considered to antagonize the estrogenic activity of EE; in this sense the third-generation progestins would counterbalance EE less than second-generation progestins (*vide infra;* see Section X).

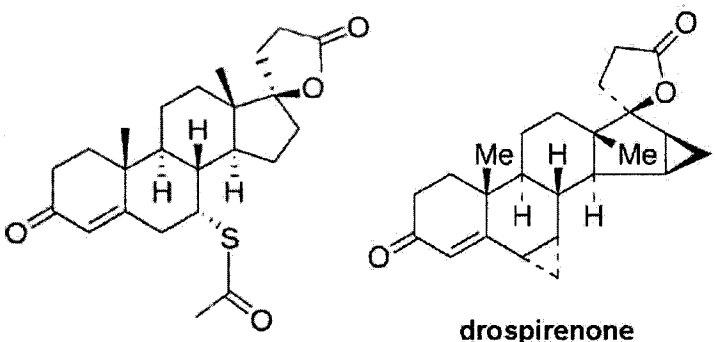
53. The newer fourth-generation progestins (*e.g.*, drospirenone) have no androgenic activity, but do display antiandrogenic activity and antimineralocorticoid activity (Sitruk-Ware & Nath, *Contraception* 82: 410-417, 2010; Rice & Thompson, *US Pharmacist* 6: 62-70, 2006; Sitruk-Ware, *Climacteric* 8(Suppl 3): 4-12, 2005; Nath & Sitruk-Ware, *Climacteric* 12(Suppl 1): 96-101, 2009; Sitruk-Ware, *Maturitas* 61: 151-157, 2008). Drospirenone (chemically related to 17α-spironolactone) is not only pharmacologically distinct, but also structurally distinct, from previous generations of progestins (Burkman *et al.*, *Contraception* 84: 19-34, 2011; Rowlands, *J. Fam. Plan. Reprod. Health Care* 29: 13-16, 2003; Thorneycroft, *J. Reprod. Med.* 57(Suppl 11): 975-980, 2002; Schindler *et al.*, *Maturitas* 61: 171-180, 2008; Sitruk-Ware, *Human Reprod. Update* 12: 169-178, 2006). Because it has a markedly distinct pharmacology (see Chart 49 above and further discussion below), it is quite possible *a priori* that the beneficial and/or adverse effects of combination oral contraceptives containing drospirenone as progestin could be different from those of COCs containing older progestins.

IX. Pharmacology of Drospirenone

54. Drospirenone is a distinct progestin by its chemical structure as well as by its pharmacology (*vide supra*). It is derived from the diuretic drug spironolactone (see figure below), and that structure contributes some of drospirenone's biological activities.

55.

Drospirenone is derived from spironolactone. Note similarity in upper right ring of each drug.



17α-spironolactone drospirenone

56. Spironolactone is an antimineralocorticoid, meaning it antagonizes the action of the natural hormone aldosterone at the mineralocorticoid receptor. This activity means that spironolactone is a diuretic (enhances urine production) of a particular sort. Specifically, spironolactone is a "potassium-sparing" diuretic that acts on the kidney to cause potassium to be retained in the blood rather than being eliminated in the urine. Thus spironolactone can lead to blood potassium levels which are higher than normal, a state called "hyperkalemia".

57. It soon became clear in human trials that drospirenone is indeed a diuretic, producing increased urine flow in a dose-dependent manner (Bayer Study 9274). The risk of hyperkalemia in patients using DRSP is a reasonable concern, and was evaluated in several trials. The labels for *Yasmin*® and *Yaz*® note that DRSP is more potent than spironolactone at producing this effect, and contain a warning about hyperkalemia. Patients at risk (e.g., because of poor renal, hepatic, or adrenal function) are cautioned to avoid drospirenone-containing COCs, as are patients taking other medications that could also induce hyperkalemia. The mechanism by which DRSP affects potassium is, like that of spironolactone, via the aldosterone pathway, where DRSP shows antimineralocorticoid activity. This pharmacological activity is one that makes DRSP distinct from most other progestins. In theory, the antimineralocorticoid diuretic activity of DRSP could ameliorate the symptoms of water retention and weight gain that appear during the menstrual cycles of some women, but such amelioration of these symptoms does not appear to be the case in practice (Gallo *et al.*, *Cochrane Database Syst. Rev.* 1: CD003987, 2009; Lawrie *et al.*, *Cochrane Library* 5:1-124, 2011, and references therein).

58. The other pharmacological activity of drospirenone that differentiates it from nearly all other progestins used in COCs is that DRSP is antiandrogenic. Older progestins tend to be androgenic, mimicking androgens by acting at agonists at androgen receptors (see Section VIII above). A few older progestins are not androgenic. DRSP, however, is antiandrogenic, meaning it antagonizes the actions of androgens at their receptors. This makes it almost unique

among the progestins used in COCs. In theory, the antiandrogenic activity of DRSP could help patients suffering from acne, seborrhea or hirsutism, but amelioration of these symptoms does not appear to be the case in practice (Arowojolu *et al.*, *Cochrane Database Syst. Rev.* 1: CD004425, 2009; Lawrie *et al.*, *Cochrane Library* 5:1-124, 2011, and references therein).

59. The antiandrogenic activity of DRSP raises the possibility that it could act differently than other progestins found in COCs with respect to opposing some of the pharmacological activities of the estrogen ethinyl estradiol. This topic is discussed further in the next section of this report.

X. Interactions of progestins and estrogens

60. While an estrogen alone can suppress ovulation, there are good pharmacological and medical reasons why estrogens in oral contraceptives are not administered alone. First, the combination of an estrogen and a progestin is more potent than either alone at suppressing GnRH (and other hypothalamic/pituitary hormones) and thus ovulation. Thus the combination allows lower doses of both drugs, than of either if given alone, while maintaining efficacy. Second, the co-administration of a progestin can make use of an estrogen safer. Use of "unopposed" estrogen promotes endometrial growth and differentiation, and early studies of estrogen-dominant contraceptives demonstrated that these OCs increase the risk of endometrial hyperplasia and endometrial cancer. This adverse activity of estrogens is opposed by progestins (to varying degrees within the progestin family), so a progestin is always co-administered with an estrogen in oral contraceptives, at least for women with a uterus (Golan *et al.*, 2012, *op. cit.*; Katzung *et al.*, 2009, *op. cit.*).

61. The concept of some progestins opposing the action of estrogens is not limited to effects on endometrium. Consider the case of hepatic protein synthesis. In this case, progestins antagonize the stimulatory effects of EE on biosynthesis of proteins such as SHBG, and different progestins antagonize this EE activity to different extents. Thus while treatment with the estrogen EE alone raises serum levels of SHBG (2 to 5 fold, dose-dependent), treatment with the progestins desogestrel or levonorgestrel alone resulted in a decrease of SHBG by 50% (Kauppinen-Mäkelin *et al.*, *Clin. Endocrinol.* 36: 203-209, 1992) and the progestin gestodene alone reduces SHBG by 25% (Steingold *et al.*, *J. Clin. Endocrinol.* 62: 761-766, 1986). Levonorgestrel, however, antagonized the EE-induced rise in SHBG, while desogestrel did not (Jung-Hoffmann *et al.*, *Contraception* 38: 593-603, 1988). While the androgenic progestins desogestrel and gestodene appear to reduce the EE-induced rise of SHBG, the antiandrogenic progestins dienogest and nomegestrol do not influence the same EE effect (Kuhl, *Drugs* 5: 188-215, 1996).

62. Drospirenone is notably antiandrogenic, and DRSP-containing COCs such as *Yasmin*® significantly increase SHBG concentrations (*e.g.*, >4-fold in Bayer's Study A40196). The same could be said of cyproterone acetate, another antiandrogenic progestin. I note that these two progestins were among those showing the highest risk of VTE in the epidemiology studies of van Hyckama Vlieg *et al.* (2009) and Lidegaard *et al.* (2009). The latter and other epidemiology studies are discussed in more detail below (see Section XII).

63. The concept of some progestins opposing the action of estrogens also extends to thrombosis. The prothrombotic effects of estrogens like EE in hormonal contraceptives are

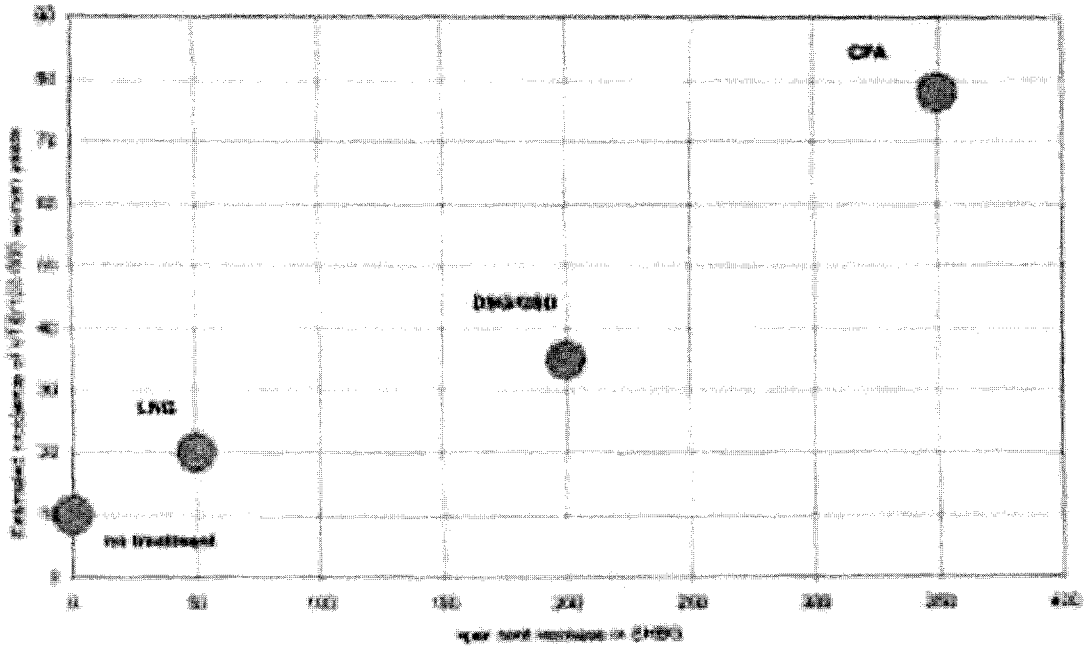
related to changes in hemostatic parameters. But progestin-only OCs cause changes in hemostatic parameters that are in many cases the opposite of those caused by EE or COCs (Tans *et al.*, 2000, *op. cit.*), suggesting an antithrombotic effect of progestins counteracting the prothrombotic effect of estrogen (Battaglioli & Martinelli, 2007, *op. cit.*). As discussed elsewhere in this report (Section XII), it is clear from epidemiology that third-generation progestins carry a higher risk of thrombosis in COCs than second-generation progestins. That may be due to a lower antiestrogenic and lower androgenic effect of third-generation vs. second-generation progestins (Tans *et al.*, 2000, *op. cit.*; Battaglioli & Martinelli, 2007, *op. cit.*). It is notable in this context that drospirenone-containing COCs cause hemostatic changes similar to those caused by COCs containing the third-generation progestin desogestrel (Kluft *et al.*, *Contraception* 73: 336-343, 2006). In the latter study, the DRSP-containing COC increased the prothrombotic proteins fibrinogen and Factor VII, and decreased the antithrombotic protein, protein S.

64. Specific examples of progestin antagonism of ethinyl estradiol's prothrombotic effect include the following. Elevation of Factor II and Factor VII was induced by 30 µg/d EE + desogestrel, but not by 30 µg/d EE + levonorgestrel. Increases in Factor V and platelet aggregation (prothrombotic) were seen with EE + levonorgestrel but not with EE + desogestrel (Prasad *et al.*, *Contraception* 39: 369-383, 1989).

65. Bayer studies A40196, A09151 and A25966 were all consistent with a prothrombotic activity of DRSP-containing COCs. Increases in fibrinogen, Factor VII and APC resistance were consistent across all three studies. These studies are discussed in more detail below (Section XIX).

66. The prothrombotic effect of COCs is sometimes considered to be a function of the "total estrogenicity" of the particular preparation (Odlind *et al.*, *Acta Obstet. Gynecol. Scand.* 81: 482-490, 2002; Tchaikovski & Rosing, *Thromb. Res.* 126: 5-11, 2010). Thus the prothrombotic effect would be related to estrogen dose, other factors being equal. But the estrogenicity of a given dose of EE would decrease with increasing dose of an androgenic progestin (such as levonorgestrel). The third-generation progestin are less androgenic and therefore less effective (than second-generation progestins) at counterbalancing the estrogenic effects of EE, and COCs containing them are therefore more prothrombotic and carry a higher thrombotic risk (Odlind *et al.*, *Acta Obstet. Gynecol. Scand.* 81: 482-490, 2002; Kemmeren *et al.*, *Blood* 103: 927-933, 2004). Since SHBG levels (discussed above) also reflect total estrogenicity, and since levels of SHBG correlate positively with COC-induced thrombotic risk (Odlind *et al.*, *Acta Obstet. Gynecol. Scand.* 81: 482-490, 2002; Tchaikovski & Rosing, *Thromb. Res.* 126: 5-11, 2010), increased SHBG levels have been proposed as a surrogate marker for VTE risk in COC users (Odlind *et al.*, *Acta Obstet. Gynecol. Scand.* 81: 482-490, 2002). See the figure below.

67.



Estimated incidence of VTE (Y-axis) per 100,000 woman-years vs. reported average increase in SHBG (X-axis) in untreated women and in women using COCs with 30-35 µg/d ethinyl estradiol (EE) with levonorgestrel (LNG), desogestrel/gestodene (DSG/GSD) or cyproterone acetate (CPA). From Odlind *et al.*, *Acta Obstet. Gynecol. Scand.* 81: 482-490, 2002.

68. The proposition that SHBG could be a surrogate marker for VTE risk (Odlind *et al.*, *Acta Obstet. Gynecol. Scand.* 81: 482-490, 2002) was vigorously attacked in a Bayer document (*SHBG and VTE, Reply to the publication of Odlind et al., Acta Obstet Gynecol Scand 2002: 81 482-490*; BHCPYAZ012116973) whose author and date were not noted. A commentary (Stanczyk & Grimes, *Contraception* 78: 201-203, 2008) arguing against SHBG as a surrogate marker for VTE was co-authored by David Grimes, a member of a board (INAS Advisory Counsel) financially supported by Bayer (BSPYAZ004158427). In the commentary (*Contraception*, 2008, *op. cit.*), Dr. Grimes argued against the use of SHBG as a surrogate marker for VTE risk, partly on the basis of the EURAS study (Dinger *et al.*, *Contraception* 75: 344-354, 2007; discussed in Section XII of this report). The European Active Surveillance Study was an epidemiology study, funded by Bayer, that compared VTE rates in women using DRSP-containing COCs to VTE rates in women using COCs containing other progestins and concluded no higher risk in the former group vs. the latter. In a meeting of the INAS Advisory Counsel held days after submission of the commentary (*Contraception*, 2008, *op. cit.*), the group discussed as an Action Item the objective to "Establish EURAS as the "Gold Standard" of OC related VTE information". In an exchange of Letters to the Editor of *Contraception* (79: 328-330, 2009) with Dr. van Vliet *et al.*, Dr. Grimes described the EURAS study as "landmark" and "methodologically superior". Because substantial published articles (discussed in Section XII of this report) establish an increased risk for VTE among users of COCs containing third-generation progestins or DRSP, and cast doubt upon the EURAS study, I do not find Dr. Grimes' opinion persuasive.

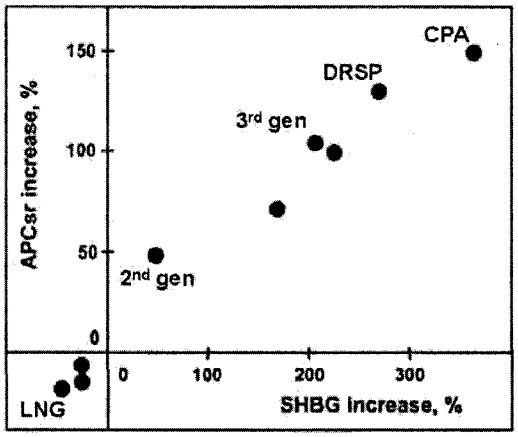
69. Regardless of whether or not SHBG may be a valid surrogate marker for VTE risk, substantial research clearly links those COCs known to increase SHBG levels and APC resistance with a greater risk of VTEs. In a randomized crossover study (van Rooijen *et al.*, *Am. J. Obstet. Gynecol.* 190: 332-337, 2004), the same group of women were treated with COCs containing levonorgestrel (a second-generation progestin) or desogestrel (third-generation) in addition to the same dose of EE. Changes in APC resistance correlated with changes in SHBG levels. In another study (van Vliet *et al.*, *Hum. Reprod.* 20: 563-568, 2005), a large group of women using various COCs were checked for SHBG levels and APC resistance. Higher SHBG levels correlated with higher APC resistance, and women using COCs containing cyproterone acetate, gestodene or desogestrel showed higher APC resistance and higher SHBG levels than those using COCs containing levonorgestrel.

70. Tchaikovski & Rosing (*Thromb. Res.* 126: 5-11, 2010) have reviewed the effects of third- vs. second-generation progestins on a number of prothrombotic and antithrombotic proteins. They have also noted a remarkable linear correlation (r=0.99) between APC sensitivity ratio (APCsr), which is highly predictive of VTE risk, and the SHBG increase induced by various hormonal contraceptives (see figure below). I note that the increase in SHBG induced by *Yasmin*® and other DRSP-containing COCs is very high, >300% in some Bayer studies (discussed below). For example, in Bayer Study A40196, a 3-week treatment with *Yaz*® raised SHBG levels by 385%, and a 14-week treatment raised SHBG levels by 513%. Such a large increase in SHBG levels (well off the axes of the graph below) constitutes a significant cause for concern about the safety of these products.

71.

Increasing APCsr correlates with increasing SHBG for COCs

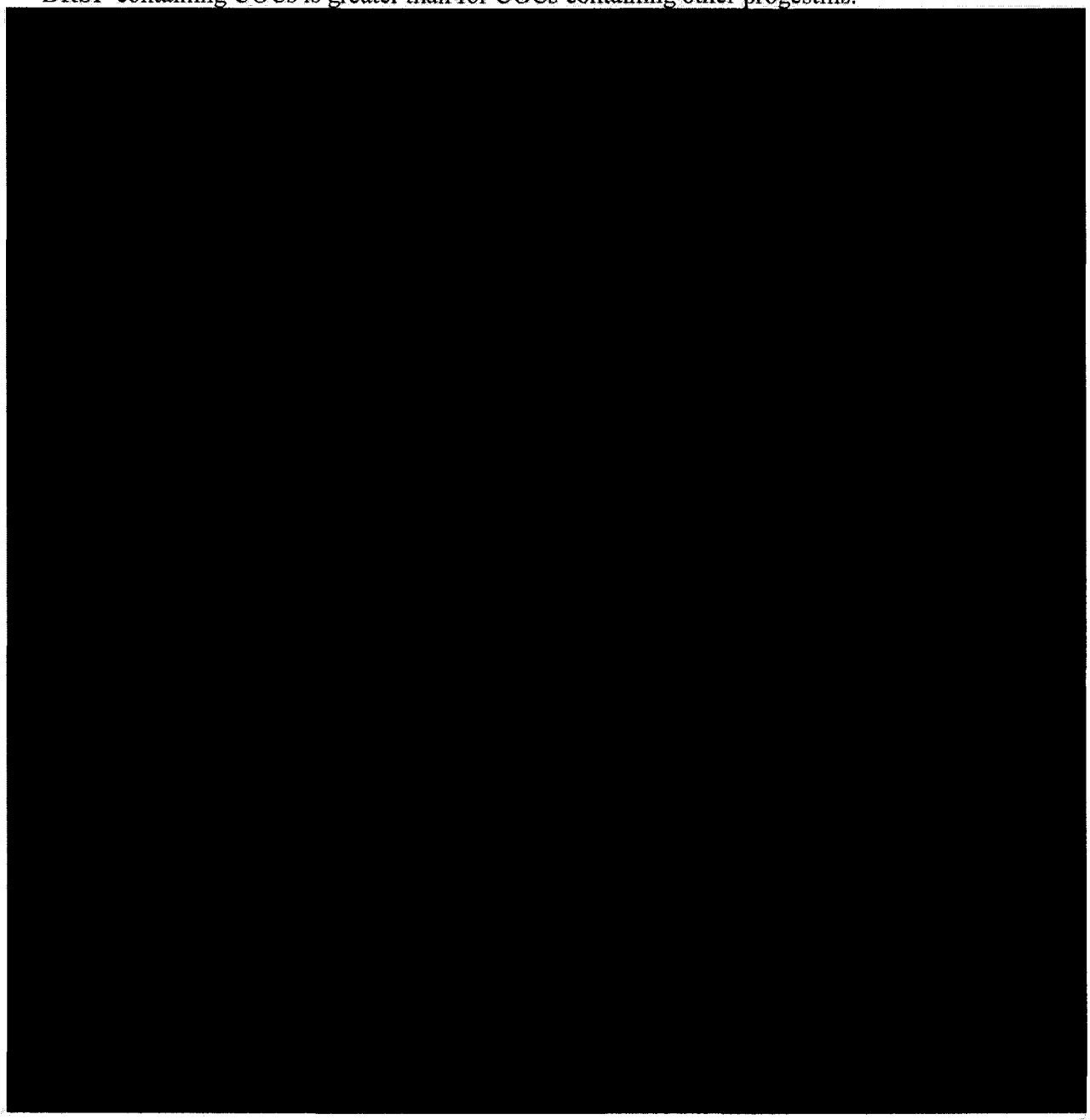
Activated Protein C sensitivity ratio is closely associated with (and proposed as a surrogate for) VT risk. APCsr correlates closely with SHBG increase for various contraceptives. Tchaikovski & Rosing, *Thromb. Res.* 126: 5-11, 2010.



LNG=levonorgestrel only, DRSP=drospirenone, CPA=cyproterone acetate

72. A Bayer document (*Sex Hormone Binding Globulin*, BHCPYAZ028783462-473) reviewed the effects of various progestins on the EE-induced increase in SHBG levels, and the

effects of DRSP-containing COCs on SHBG. As discussed above, progestins with androgenic activity (*e.g.*, the first-generation compound norethindrone or the second-generation compound levonorgestrel) antagonize the increase in SHBG caused by EE more than progestins with slight androgenicity (*e.g.*, the third-generation compounds desogestrel and gestodene) or progestins with antiandrogenic effects (*e.g.*, the fourth-generation compounds drospirenone and cyproterone acetate). Drospirenone is the only progestin available (in COCs approved in the US) that is significantly antiandrogenic at therapeutic doses. Thus the increase in SHBG by DRSP-containing COCs is greater than for COCs containing other progestins.



XI. Risks of Hormonal Contraceptives

76. It is a principle of pharmacology that the incidence and severity of adverse effects of drugs is dose-dependent, and it is a principle of therapeutics that physicians should employ the lowest dose of a drug that produces the desired effect. This general principle is particularly applicable to hormonal contraceptives, where the dose-dependent risks and adverse effects of the estrogens have been well known for many years (*e.g.*, Inman *et al.*, *Brit. Med. J.* 2: 203-209, 1970; Meade *et al.*, *Brit. Med. J.* 280: 1157-1161, 1980). This recognition has led to the significant lowering of estrogen doses in OCs over time (from 150 μg/day in the 1960s to 10-35 μg/day today), and the withdrawal from the US market in 1988 of OCs containing more than 50 μg/day. This progression toward lower estrogen doses is shown in the figure below.

77. History of estrogen dose in OCs.

Later generation OCs have less estrogen and hence lower risk for thromboembolism.

- **1960s 150 μg/day estrogen** (mestranol)
- **1970s ≥50 μg/day estrogen** (ethinyl estradiol)
- **2000s ≤35 μg/day estrogen** (ethinyl estradiol)

OCs with >50 μg/day estrogen were withdrawn after FDA action in 1988. 16

78. I find it striking how often COC labels and pharmacology textbooks remind physicians to use the lowest possible dose of estrogens versus how often the reminder is stated with other drugs. For example, consider the following quotes from the relevant chapter of Goodman & Gilman (2006, *op. cit.*). "Regardless of the specific agent or regimen, … therapy with estrogens should use the lowest dose and shortest duration necessary to achieve an appropriate therapeutic goal" (page 1554). "Treatment should … begin with preparations containing the minimum dose of steroids that provides effective contraceptive coverage" (page 1567). "*Regardless of the specific drug(s) selected, treatment should use the minimum dose and duration for the desired therapeutic endpoint*" (page 1568). [Similar "class warnings" appear on the labels of OCs; *vide supra.*] A product that delivered a higher dose of estrogen than most other available OCs would be a poor choice, especially for a new user, if lower dose OCs were available and efficacious. A product that delivered a higher daily dose of estrogen than the

equivalent of a 50 µg/day OC after 1988 would be an extremely poor choice and would expose the patient to unnecessary risk.

79. The incidence of venous thromboembolism ("VTE") in women of childbearing age is low, and varies based on age and other factors. The EMA Committee for Proprietary Medicinal Products (*Advice and Information*, 28 September 2001) estimated the incidence at about 5 per 100,000 women-years in healthy non-pregnant women not using COCs. Although the absolute VTE risk is low, approximately 100,000 to 1,000,000 women suffer from VTE each year worldwide. Since hormonal contraceptives are in widespread use, by at least 100 million women in developed countries and 9% of women worldwide (UN Department of Economic and Social Affairs, World Contraceptive Use 2007), even a fairly small increase in the incidence of adverse effects would affect a significant number of patients. The incidence of VTE in users of COCs is about 3-5 times higher than in non-users, and use of COCs is the most frequent risk factor for VTE in women of childbearing age (Battaglioli & Martinelli, *Curr. Opin. Hematol.* 14: 488-493, 2007). Use of COCs is responsible for a large part, if not the majority, of venous thromboses in that group (Tchaikovski & Rosing, *Thrombosis Res.* 126: 5-11, 2010). VTE risk is lower with lower doses of estrogens, and COCs have become safer over time as estrogen doses have declined. But the nature of the progestin is also related to VTE risk. The pharmacology of different progestins was discussed above. The epidemiology of thromboembolic risk of COCs as a function of the progestin will be discussed below.

XII. Epidemiological studies of COC risk

80. As a pharmacologist, I am interested in the adverse effects of drugs in the human population, and review such literature as part of my profession. Here I briefly summarize the results of published studies I have read about the thromboembolic risk of various oral contraceptives, focusing on the progestin component. Recall that there are essential pharmacological differences (*vide supra*) between different generations of progestins. One of the differences is in the androgenicity of the progestins.

81. As a group, the third-generation progestins (*e.g.*, gestodene, desogestrel) have less androgenic efficacy than the second-generation progestins (*e.g.*, levonorgestrel, norgestrel, norgestrione). And as a group, users of OCs containing the third-generation progestins have higher incidence of VTEs than users of OCs containing second-generation progestins. The difference is significant. While patients on second-generation progestin OCs showed about 10 additional VTE cases per 100,000 woman-years compared to non-users, patients on third-generation progestin OCs showed about 25 additional VTEs (Battaglioli & Martinelli, 2007, *op. cit.*; Heinemann *et al.*, *Eur. J. Contracept. Reprod. Health* 4: 67-73, 1999; Hannaford, *Brit. Med. Bull.* 56: 749-760, 2000). The magnitude of the increased risk varied from study to study (WHO Collaborative Study, *Lancet* 346: 1582-8, 1995; Jick *et al.*, *Lancet* 346: 1589-1593, 1995; Spitzer *et al.*, *Brit. Med. J.* 312: 83-88, 1996), and was at one time a matter of debate, but a strong consensus that third-generation progestins carry a higher risk of VTEs than second-generation progestins emerged (*e.g.*, Tchaikovski & Rosing, *Thromb. Res.* 126: 5-11, 2010). A meta-analysis (Kemmeren *et al.*, *Br. Med. J.* 323: 131-134, 2001) of twelve studies carried out before 1995 reported an overall adjusted odds ratio for third-generation vs. second-generation progestins of 1.7 (95% CI 1.4 to 2.0). Recent studies which carefully controlled potentially confounding variables (van Hylckama Vlieg *et al.*, *Brit. Med. J.* 339: b2921, 2009; Jick *et al.*,

Contraception 73: 566-570, 1996) clearly demonstrated increased VTE risk for the third-generation progestin desogestrel vs. the second-generation progestin levonorgestrel.

82. While there are other risk factors for VTE besides OC use, these seem unlikely to confound results comparing third- and second-generation progestins. For example, there are inherited thrombophilias that raise the risk of patients who inherit them significantly compared with normal patients, but the risk of thrombosis in carriers of thrombophilias is still increased by OC use. The risk factors of thrombophilia and OC use appear to be multiplicative; *i.e.*, greater than additive (Battaglioli & Martinelli, 2007, *op. cit.*; Tchaikovski & Rosing, 2010, *op. cit.*).

XIII. Epidemiological studies of COCs containing drospirenone

83. Soon after the launch of the first drospirenone-containing COC, *Yasmin*®, in 2001, a series of reported cases (Sheldon, *Brit. Med. J.* 324: 869, 270, 2002; van Grootheest *et al.*, *Brit. Med. J.* 326: 257, 2009) of venous thrombosis raised early concerns about increased risk associated with this new progestin, and Dutch general practitioners were warned about DRSP by their professional association (Sheldon, *op. cit.*). A report (Pearce *et al.*, *Brit. J. Clin. Pharmacol.* 60: 98-102, 2005) of thirteen cases of DVT or PE in women using *Yasmin*® in the UK (from questionnaires sent to prescribing doctors) concluded that this drospirenone-containing COC is associated with thromboembolism. The latter publication is sometimes called the PEM study.

84. A few years later, two studies sponsored by *Yasmin*®'s manufacturer, at that time Schering, reported results (Dinger *et al.*, *Contraception* 75: 344-354, 2007; Seeger *et al.*, *Obstet. Gynecol.* 110: 587-593, 2007). Both failed to detect a difference in thrombotic risk for OCs containing drospirenone vs. OCs containing levonorgestrel. The former is sometimes referred to as the EURAS study, the second as the Ingenix (or i3) study.

85. Later, the same group published a case-control study (again sponsored by *Yasmin*®'s manufacturer, Bayer Schering AG) based on the patient charts from primary care physicians in Germany (Dinger *et al.*, *J. Fam. Plann. Reprod. Health Care* 36: 123-129, 2010). In this study, the authors found an elevated risk of 2.4 (95% CI 1.8 to 3.2) for COC use vs. never use, but no elevated risk for users of dienogest- or drospirenone-containing COCs vs. users of levonorgestrel-containing COCs. These results are not in agreement with several epidemiology studies from other groups (*vide infra*). The Dinger group studies are notable for a very high incidence rate of VTEs compared with the generally accepted age-adjusted incidence and for few exclusions of high risk demographics (*e.g.*, older patients and pregnant patients). This methodology would be expected to lead to a higher baseline incidence, making the effects of COC use more difficult to detect and quantify.

86. Additional epidemiology studies of COCs containing drospirenone (DRSP) and other fourth-generation progestins vs. COCs containing older progestins have begun to appear, now that the former drugs have been in use long enough to gather data from large numbers of users. The following studies were supported by public entities.

87 Lidegaard *et al.* (*Brit. Med. J.* 339: b2890, 2009) reported on the incidence of venous thromboembolism in a cohort study based on 10.4 million woman-years. [This paper is

sometimes called the Danish study.] Consistent with many other studies, they found that users of COCs were at higher risk of VTE than non-users, and that higher estrogen doses were associated with higher risk, and that COCs containing third-generation progestins conveyed higher risk than COCs containing second-generation progestins. Again consistent with other studies, COC users were at highest risk in the first year of use, with a rate ratio of 4.17 (95% CI 3.73 to 4.66) compared to non-users; the risk ratio for >four years of use was 2.76 (95% CI 2.53 to 3.02). When the group of first year COC users was restricted to COCs containing 30-40 µg/d estrogen and parsed by the progestin present, patterns of different VTE risks were observed for different progestins. Some of the findings are presented in the figure below.

88.

Risk of VTE for COCs containing different progestins[1]

Progestin (gen)	Rate Ratio[2]
Norethindrone (1st)	2.81 (1.66-4.77)
Levonorgestrel (2nd)	1.91 (1.31-2.79)
Norgestimate (3rd)	3.37 (2.38-4.76)
Drospirenone (4th)	7.90 (5.65-11.0)

[1]Lidegaard *et al.*, *BMJ* 339: b2890, 2009. Denmark, 10.4 million woman-years.
[2]Rate ratio (95% CI) in 1st year of use, adjusted for age, calendar year, education; vs. OC non-users (=1.00). All COCs contained 30-40 µg/d estrogen.

89. Among current users of COCs with 30-40 µg/d estrogen, the adjusted rate ratio in this study for drospirenone vs. levonorgestrel was 1.64 (95% CI 1.27 to 2.10; statistically significant). Upon a reanalysis requested by EMA and supported by Bayer, the data were found to support an enhanced risk of DRSP over levonorgestrel of "about 2" at 30 µg/d EE (Lidegaard *et al.*, Supplementary Analysis of 29 March 2011, BHCPYAZ029109807). The majority of the Steering Committee overseeing the reanalysis concluded "that the work carried out by Dr. Lidegaard has been done diligently and that his final report provides sufficient information for the MEB to obtain a reliable picture of the relation between oral contraception and venous thrombosis in Denmark during the last decade."

90. My reading of the reanalysis of the Danish study is that while some of the data has been parsed differently, the fundamental numbers have not significantly changed. For example, consider the chart above, addressing VTE risk in the first year of use, from *BMJ* 339: b2890, 2009. The relative risks for less than one year of use for DRSP and LNG (vs. COC non-users) were 7.90 and 1.91, respectively. In the reanalysis, the same relative risks were 8.46 and 2.04, respectively (see Table 14 of reanalysis). Thus the ratio of the relative risks for DRSP vs. LNG was 4.14 in *BMJ* 2009 and 4.15 in the reanalysis.

91. A smaller (1524 patients, 1760 controls) case-control study (van Hylckama Vlieg *et al.*, *Brit. Med. J.* 339: b2921, 2009) from the Netherlands appeared about the same time. This study (the MEGA case-control study) gave results consistent with the Danish study in that the very same rank order of adjusted risk (drospirenone > norgestimate > norethindrone > levonorgestrel) was found, but differences were not statistically significant.

92. A paragraph discussing both the van Hylckama Vlieg *et al.* (2009) study and the Lidegaard *et al.* (2009) study was incorporated into the *Yasmin*® and *Yaz*® labels in April 2010.

93. A recent nested case-control study (Parkin *et al.*, *Brit. Med. J.* 340: d2139, 2011) based on the UK General Practice Research Database compared the risk of OCs containing drospirenone vs. OCs containing levonorgestrel as progestin. In contrast to the Bayer-supported studies above, Parkin *et al.* used a study population without major risk factors for VTE, eliminating such potential confounds as the influence of such risk factors on prescribing behavior and the dilution of effect due to high baseline incidence (Jick *et al.*, *Lancet* 352: 1767-1770, 1998; Rothman & Pool, *Intl. J. Epidemiol.* 17: 955-959, 1988). Since the large majority of OC users do not have major risk factors for VTE other than OC use, a study designed in this way should have greater validity for the group of "normal" users of COCs. Parkin *et al.* found a statistically significant increased risk of nonfatal VTE for COCs containing drospirenone over those containing levonorgestrel. The adjusted (for body mass index) matched odds ratio was 3.3 (95% CI 1.4 to 7.6) when missing values for BMI and smoking status were imputed. When cases and controls whose BMI and smoking status were missing were excluded completely, the adjusted matched odds ratio was 2.9 (95% CI 1.1 to 7.6). Both odds ratios were statistically significant.

94. Parkin *et al.* note that their findings of higher VTE risk in users of drospirenone vs. levonorgestrel OCs is consistent with the observation that activated protein C resistance (prothrombotic) was higher in the users of drospirenone OCs (van Vliet *et al.*, *J. Thromb. Haemost.* 2: 2060-2, 2004; Tchaikovski *et al.*, *Thromb. Haemost.* 98: 1350-6, 2007). Parkin *et al.* close by saying:

> "This study adds to emerging evidence that use of oral contraceptives containing drospirenone is associated with a higher risk of venous thrombosis than are preparations containing levonorgestrel. Perhaps now is the time for a systematic review on this topic. In the meantime, as no clear evidence exists to show that the use of the drospirenone pill confers benefits above those of other oral contraceptives in preventing pregnancy,[25] treating acne,[26] alleviating premenstrual syndrome,[27] or avoiding weight gain,[28] prescribing lower risk levonorgestrel preparations as the first line choice in women wishing to take an oral contraceptive would seem prudent."

As evidence that drospirenone confers none of the above benefits over other OCs, the authors cite: [25]Maitra *et al.*, *Cochrane Database Syst. Rev.* 1: CD004861, 2009; [26]Arowojolu *et al.*, *Cochrane Database Syst. Rev.* 1: CD004425, 2009; [27]Lopez *et al.*, *Cochrane Database Syst. Rev.* 1: CD006586, 2009; [28]Gallo *et al.*, *Cochrane Database Syst. Rev.* 1: CD003987, 2009.

95. Another recent nested case-control and cohort study based on US claims (PharMetrics) data also compared the risk of OCs containing drospirenone vs. levonorgestrel. As with the

UK study, patients with non-idiopathic risk factors for VTE were excluded from the analysis. Consistent with the above UK study, the US study (Jick & Hernandez, *Brit. Med. J.* 340: d2151, 2011) found a statistically significantly higher risk of non-fatal VTE in users of drospirenone-containing COCs than in users of levonorgestrel-containing COCs. The age-adjusted incidence rate ratio was 2.8 (95% CI 2.1 to 3.8). For users of COCs containing 20 μg/d of ethinyl estradiol, the same odds ratio was 3.2 (95% CI 1.8 to 5.5). When the database was parsed by age, the overall incidence of VTE increased with increasing age as expected, but the higher risk of drospirenone was statistically significant in all age groups. In patients <30 yo, the incidence rate ratio for DRSP relative to levonorgestrel was higher still at 4.6 (95% CI 2.6 to 8.2), as shown in the figure below.

96.

In patients < 30 yo, the relative risk of VTE for drospirenone vs. levonorgestrel OCs is even higher

Progestin	VTE incidence	
User Age:	15-44 yo	15-30 yo
Levonorgestrel	(1)	(1)
Drospirenone	2.8	4.6
95% CI	(2.1-3.8)	(2.6-8.2)

Incidence rate ratio for VTE in users of DRSP vs. levonorgestrel OCs. PharMetrics database of US claims. *BMJ* 340: d2151, 2011.

97. A more elevated risk in younger patients was also shown by Parkin *et al.* (*Brit. Med. J.* 340: d2139, 2011), who reported an adjusted (for BMI) odds ratio for users of DRSP-containing COCs vs. users of LNG-containing COC of 3.7 (95% CI 1.3 to 10.7) for patients <35 yo, compared with a ratio of 2.8 (95% CI 0.7 to 10.7) for patients ≥35 yo.

[redacted]

[redacted]

100. The 2011 studies from UK and US databases are summarized in the table below.

101.

VTE risk is significantly higher for drospirenone than for levonorgestrel

Progestin	VTE incidence UK	US
Levonorgestrel	(1)	(1)
Drospirenone	2.7	2.8
95% CI	(1.5-4.7)	(2.1-3.8)

Age-adjusted incidence rate ratio (and 95% CI) of VTE for databases from the UK (General Practice Research Database) and the US (PharMetrics Claims Database). *BMJ* 340: d2139, 2011; *BMJ* 340: d2151, 2011.

102. In summary, epidemiological studies (with the exception of those funded by the manufacturer of drospirenone-containing OCs), unanimously conclude that VTE risk is higher with DRSP-containing OCs than with OCs containing the second-generation progestin levonorgestrel. It is my expectation that additional epidemiological studies comparing the risks of OCs containing drospirenone with the risks of COCs containing older progestins will continue to appear in the scientific literature, and that future studies employing reasonable methodology will continue to support the conclusions of the studies done to date.

103. In May 2011, the European Medicines Agency (EMA) Pharmacovigilance Working Party completed a review of the epidemiological studies above (including some re-analysis) evaluating the association between DRSP-containing COCs and VTE (*EMA Monthly Report*, Issue 1105, 26 May 2011). They concluded: "The results from the reviewed studies have shown that drospirenone-containing COCs are associated with a higher risk of VTE than levonorgestrel-containing COCs. Data indicate that the risk for drospirenone may be similar to the risk for COCs containing desogestrel or gestodene." On 27 May 2011, EMA announced that it is updating the labels for these products.

104. On 31 May 2011, FDA reacted to the epidemiology studies above with a Drug Safety Communication informing the public about the recent *BMJ* publications (above), which are currently being assessed as part of an ongoing safety review of DRSP-containing OCs. Data from an ongoing FDA-funded study (sometimes called the Kaiser study) are expected later in 2011.

XIV. Why is the risk of DRSP-containing COCs significantly higher?

105. There are several pharmacologically plausible mechanisms by which DRSP-containing COCs could have greater prothrombotic effects than COCs containing older progestins. Each of these is discussed in more detail elsewhere in this report.

106. If the presence of 3 mg DRSP in a COC were to lead to greater bioavailability of the EE present, one would expect a higher EE AUC (*i.e.*, higher EE exposure) from that COC than from a COC containing the same EE dose with an older progestin. There is good reason to believe that DRSP does induce a greater EE exposure, significantly greater than the value indicated for EE AUC_{24} on the product label. It is well-established that higher EE dose carries a higher risk of VTE (see above). Bayer neglected to specifically examine the effects of DRSP on EE bioavailability.

107. If the presence of 3 mg DRSP in a COC were to lead to slower metabolic clearance of the EE present, one would expect a higher EE AUC (*i.e.*, higher EE exposure) from that COC than from a COC containing an older progestin. It is well-established that higher EE dose carries a higher risk of VTE. Bayer neglected to specifically examine the effects of DRSP on EE clearance.

108. DRSP-containing COCs significantly affect the levels of certain proteins synthesized in the liver, including proteins involved in the thrombosis cascade. COCs with less androgenic progestins and antiandrogenic progestins (such as DRSP) induce particularly large increases in SHBG, which is closely associated with increased APCres, APRsr, and a prothrombotic state. The same dose of EE has a larger effect on SHBG in the presence of DRSP than in the presence of LNG. Together these observations suggest that COCs with DRSP+EE are more prothrombotic than COCs containing other progestins.

109. Several of the activities and the adverse effects of EE are antagonized to different extents by different progestins. Antiandrogenic progestins such as DRSP could fail to counteract deleterious effects of EE to as great an extent as other androgenic progestins.

110. DRSP-containing COCs, for reasons probably related to their formulation, deliver a more variable dose of EE than other COCs. This observation shows clearly in the higher coefficients of variation about the means of EE AUC values. For example, at Day 21 of Cycle 1, the %CV for *Yasmin*® (according to its label) is 94%, vs. an average of about 27% for other COCs containing 30 μg/d EE. For example, at Day 21 of Cycle 1, the %CV for *Yaz*® (according to its label) is 57%, vs. an average of about 32% for other COCs containing 20 μg/d EE. Higher variability means that a larger fraction of patients will be exposed to higher EE levels, even if the means are the same. The association of higher EE levels with higher VTE risk, as discussed above, is causal.

XV. Introduction to pharmacokinetics

111. The study of how drugs are delivered and cleared, particularly as a function of time, is part of the field of pharmacokinetics, often abbreviated "PK". Thus the timecourses of drug absorption, distribution, metabolism, and elimination are quantitatively treated under PK. Informed comparison of drug delivery by different formulations or by different routes of administration requires an understanding of pharmacokinetics. PK is a well-developed branch of pharmacology, which is explained here in highly simplified form.

112. In considering pharmacokinetics, it is important to pay careful attention to the units of measure for the various pharmacokinetic quantities. Thus concentration (measured, *e.g.*, in μg/ml) is not the same as dose (measured, *e.g.*, in μg). [The Greek letter mu (μ) represents one one-millionth, or 10^{-6}.] Thus 10 hours is not the same as 10 days, but 168 hours is the same as 1 week.

113. The concentration of a drug at its target, over time, determines the magnitude of the effects (therapeutic or adverse) of that drug. This is a fundamental tenet of pharmacology. The concentration of a drug (at steady-state) at its target is directly proportional to the concentration of that drug ("[drug]") in the plasma.

114. While essentially the full amount of a drug delivered by intravenous ("IV") injection arrives in the plasma, that is rarely true of drugs delivered by other routes of administration. Sometimes only a small fraction of the total drug contained in a pill actually arrives in the plasma or at its target. Some drugs given orally can't reach the plasma at all. For example, insulin administered orally does not reach the plasma; this drug must be delivered by injection (or aerosol) to be effective.

115. There are well-defined equations relating various PK variables and parameters, and thus ways to calculate certain PK quantities from others. I will try to limit the use of equations here to the minimum necessary. I will discuss the pharmacokinetic parameters of the drugs in question as needed. These are well known. For example, ethinyl estradiol ("EE"), the estrogen in *Yasmin*®, *Yaz*®, and the vast majority of oral contraceptives, has half-life $t_{½} \approx 17$ hours, oral bioavailability $F \approx 51\%$, volume of distribution $V_d \approx 5$ L/kg, elimination rate constant $k \approx 0.04$ hr^{-1}, and clearance $Cl \approx 62$ L/hr/kg (approximate consensus values from textbooks and original literature). These numbers are for EE alone or in well-studied COCs using traditional formulations; For a novel formulation, such as EE that had been micronized (prepared as very small particles with high surface area) as in *Yasmin*®, or delivered in the form of a cyclodextrin clathrate (an inclusion complex) as in *Yaz*®, the PK parameters of EE should be rechecked. One could not assume that a change in formulation would be without effect on pharmacokinetics.

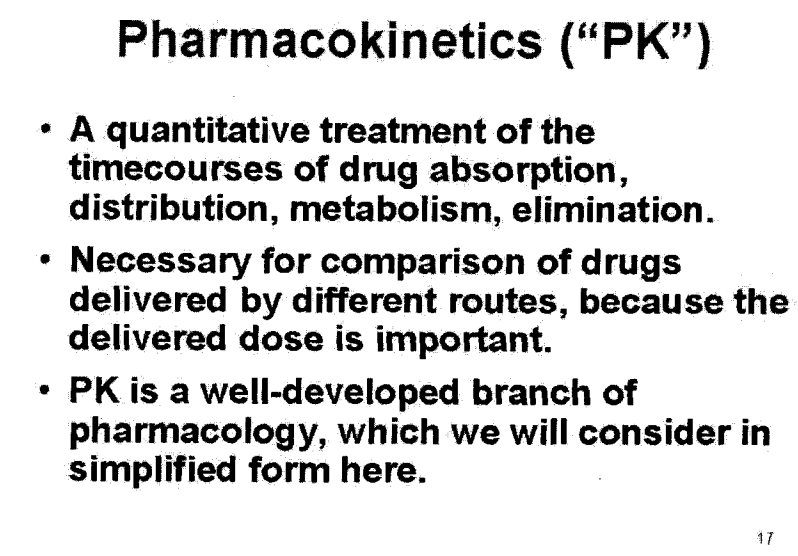
116. The half-life, $t_{½} \approx 17$ hr, means that the concentration of EE in the plasma, assuming no EE is coming in, drops by half in each 17 hour interval. Thus the concentration of EE falls from 100 to 50 pg/ml, or 50 to 25 pg/ml, or 10 to 5 pg/ml, in about 17 hours. Some variation in $t_{½}$ from patient to patient would be expected, and other drugs a particular patient is taking may affect EE half-life.

117. The oral bioavailability, $F \approx 0.51$, means that about 51% of the EE given orally reaches the plasma. Thus a typical oral contraceptive tablet containing 30 μg EE actually delivers about 51% of 30 μg = 15 μg EE to the plasma. Similarly, an oral contraceptive tablet

containing 20 μg EE actually delivers only about 51% of 20 μg = 10 μg EE to the plasma. Bioavailability can be affected by formulation, however, so different OC products containing 30 μg EE may not all deliver the same 15 μg to the plasma. It would be possible that a product containing 30 μg EE in a novel form (micronized or clathrated, for example) or differently formulated could deliver more EE than a conventional (sieved) form. Some small variation in F from patient to patient would also be expected. As far as I can tell Bayer staff did not experimentally determine either half-life of bioavailability for EE in *Yasmin*® or *Yaz*®.

118. Other PK parameters will be discussed later. Some figures summarizing the basics of pharmacokinetics follow below.

119.



120.

Fundamental Pharmacology Facts - 1

- The concentration of a drug at its target, over time, is what matters. That is proportional to the concentration of drug ("[drug]") in the plasma.
- Concentration is not the same as amount or dose, but is directly proportional to dose.
- The full amount of drug in a pill or a patch is not delivered to the plasma. Sometimes only a small fraction of the drug in a pill or patch actually arrives at the target.

1

121.

Fundamental Pharmacology Facts - 2

- There are well-defined equations relating various PK variables, and ways to calculate certain PK quantities from others.
- PK parameters for the drugs in question here are known. E.g., ethinyl estradiol (e.g., from *Ortho Tri-Cyclen*®) has half-life $t_{½} \approx 17$ hours and bioavailability $F \approx 51\%$.
- I.e., a Pill containing 35 µg EE (e.g., *Ortho Tri-Cyclen*®) actually delivers only 51% of 35 µg = 18 µg EE to the blood.

19

XVI. Pharmacokinetic Terms Defined

122. Drug concentration is measured in units of mass/volume, such as µg/ml, ng/ml, pg/ml, etc. [In contrast, drug content is measured in units of mass, such as mg, µg, ng, etc. Daily dose is measured in units of mass/time, such as mg/day, µg/day, ng/day, etc.] Concentration is

abbreviated C, or by use of square brackets; thus [EE] means concentration of ethinyl estradiol. The abbreviations n (for nano-) and p (for pico-) mean 10^{-9} and 10^{-12}, respectively.

123. The maximum or peak concentration of a drug reached during a dosing regimen is abbreviated C_{max}. The C_{max} occurs at some time (t_{max}) after a drug is administered. For a drug given IV, the time to C_{max} is typically only seconds after the injection is completed, and the peak concentration typically lasts very briefly. For a drug given orally, the time to C_{max} is typically a few hours after the drug is swallowed, and this maximum drug concentration typically lasts briefly (*vide infra*). [For a drug given transdermally, the time to C_{max} may be days after the patch is applied, and the C_{max} may continue for days.]

124. The drug concentration at steady-state is abbreviated C_{ss}. "Steady-state" (an important concept in pharmacology) means a dynamic equilibrium state where the rate of drug going into the system equals the rate of drug leaving the system. Steady-state is not reached as soon as a drug is administered; instead it may take an extended period of dosing to reach steady-state. Typically steady-state is said to have been reached at about 4-5 half-lives after a constant (whether continuous or intermittent) rate of drug delivery has been established. Steady-state and the time to reach steady-state are important in considering the PK of drugs given chronically because certain calculations are valid only at steady-state and because considering drug concentrations and effects before steady-state has been reached can be misleading. For an intermittent delivery method, such as one tablet orally every 24 hours, concentrations will vary between C_{min} (just before a new dose) and C_{max} (at t_{max} after a new dose) in repeating cycles, if the system is at steady-state. For a continuous delivery method such as IV infusion or transdermal delivery, $C = C_{max} = C_{ss}$ if and only if the system is at steady-state.

125. For drugs used chronically, C_{max} is rarely important in determining the drug's biological effects. The effects of a chronic drug are determined instead by the average C_{ss} over the period of use. For example, consider an antibiotic with a half-life of 8 hours given orally every 12 hours to treat a bacterial infection. The concentration of this drug in plasma will be highest at some time (perhaps an hour or two) after each dose is swallowed, and lowest just before the next dose is swallowed. But neither the highest concentration (C_{max}) nor the lowest concentration (C_{min}) is so important as the average C_{ss} over the period of treatment (typically days) in determining how well the drug kills the targeted bacteria.

126. The parameter that best measures total exposure to a drug (and represents total drug delivered to the bloodstream) over an interval of time is called AUC. AUC is the area under the curve of a plot of plasma drug concentration ([drug] or C) versus time (t). That is, plasma drug concentration at certain timepoints is plotted on the Y (vertical) axis against those timepoints on the X (horizontal) axis. The units of AUC are units of concentration (Y-axis) times time (X-axis), such as μg-hr/ml or ng-hr/ml. AUC usually has following subscripts to indicate the period of time over which the area under the curve is determined. Thus $AUC_{0\text{-}24h}$ means AUC for the first 24 hours following the start of drug administration at time zero, while $AUC_{0\text{-}168h}$ means AUC for the first full week following the start of drug administration.

127. Definitions of these PK terms are summarized in the figures below.

128.

Pharmacokinetics Terms - 1

- C_{max} – maximum or peak Concentration of drug. "[drug]" means drug concentration.
- C_{ss} – Drug Concentration at steady-state (amount going in = amount going out). It may take quite some time after the start of drug administration to reach steady-state.
- Drug concentration is measured in ng/ml, pg/ml, mg/l, etc. In contrast drug content is measured in mg, µg, ng, etc. Daily dose is measured in mg/day, µg/day, etc.

20

129.

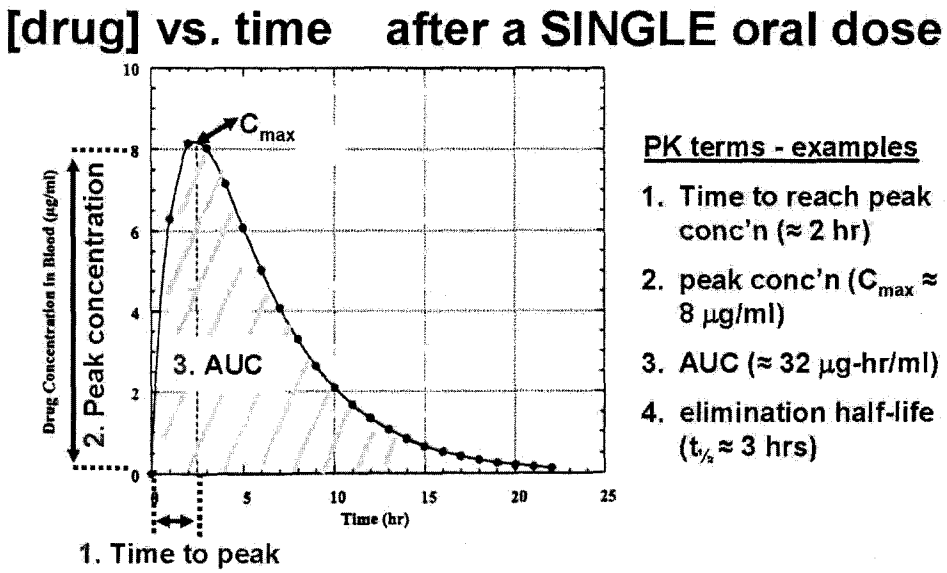
Pharmacokinetics Terms - 2

- AUC – Area under the curve of a plot of [drug] vs. time. AUC over a given time interval represents the total amount of drug delivered over that time interval.
- $t_{½}$ – Drug elimination half-life. Time for half of the drug present to be eliminated. Time for drug concentration to drop from 100 to 50, or 50 to 25, or 60 to 30, or 10 to 5, etc. $t_{½}$ is typically in units of hours.
- Examples follow …

21

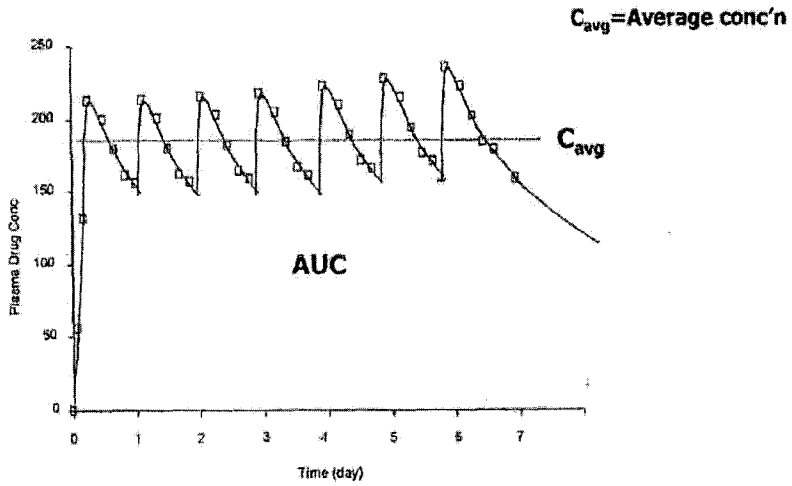
130. The following two figures serve as examples to illustrate the points discussed above about PK and the timecourses of drug delivery by different administration routes.

131.



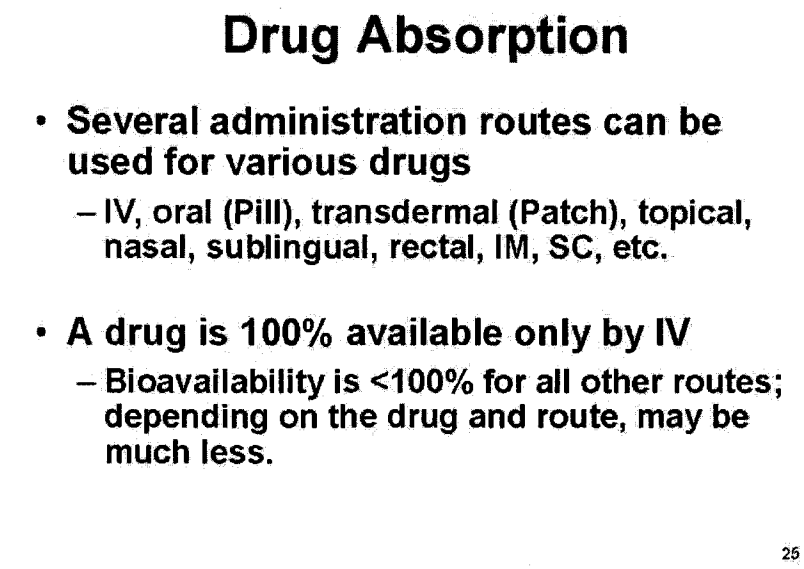
132.

[drug] vs. t – multiple oral dosing



133. To discuss the quantitative aspects of dosing, I must introduce the concept of bioavailability. This parameter is discussed further below, but in brief, bioavailability is a measure of how much of a given dose of a given drug administered in a given way actually reaches the bloodstream. Of course, if the drug is directly injected to a vein, the full dose reaches the bloodstream by definition. But for other routes, bioavailability is almost always less than 100%. For some drugs administered by some routes, bioavailability may be zero. For example, insulin given orally has zero bioavailability because it is destroyed in the gut; this drug must be given by some other route. Some key points about oral bioavailability (called F by pharmacologists) are described in the figure below.

134.



XVII. Averaging values within datasets. What to do with missing measurements?

135. In the world of experimental biology, datasets are not always complete. Consider, for example, an experiment to determine the AUC of a drug in a clinical trial. AUC, the pharmacokinetic parameter that most accurately reflects total patient exposure to a drug, must be determined for any drug that will be administered to humans. AUC is the area under the curve of a plot of drug concentration in plasma of volunteers vs. time, as shown above in Figure 131. So blood would have to be drawn from each volunteer at various times (a dozen or more timepoints) after the drug is administered, and the concentration of drug determined at each timepoint, in order to construct a curve and determine AUC for that volunteer. That same process would have to be carried out for dozens or hundreds of volunteers to determine an average AUC for the drug in the part of the human population represented by the volunteer group.

136. Ideally, all the patients would have successful blood draws at the appropriate time points, and drug concentration in every blood sample would be successfully measured by the techniques available, and for every patient there would be an accurate measurement at every timepoint. In that ideal case, the average concentration at a particular time would be the simple arithmetic mean of the values for all the individual patients, and a standard deviation would be calculated based on one value for each volunteer. In that ideal case, there would be enough valid concentration-time points to accurately determine the AUC for each patient.

137. But in the real world of determining an AUC, not every blood draw will be done at the right time for every patient, and not all the drug concentration determinations will be good

measurements. Some measurements, for example, might fall outside the valid measuring range of the drug assay being used to determine concentration. It essential to handle the dataset in a rigorous and scientifically acceptable manner. The most rigorous way to handle missing measurements is to simply leave them out of the calculations, and average the remaining good measurements. The wrong way to handle missing measurements is to arbitrarily assign either high or low values to the measurements that are missing or out of range, because the latter method would lead to systematically, inaccurately higher or lower average values, respectively.

138. I will illustrate with an example of a right and wrong way to average data, to demonstrate how a wrong (and unjustified) method can lead to a misleading report. Suppose there were 11 patients in a study (that would be a very small number for most clinical trials), and each was to have a blood draw at 12 timepoints for determination of drug concentration. Later all the concentrations and times would be used to determine AUC for each volunteer and the average AUC for the group. Suppose the measuring range for the drug concentration assay was 25-500 pg/ml. That is, values outside that range could not be measured accurately due to limitations of the drug assay itself. The lower limit for measurement (here 25 pg/ml) is often called the lower limit of quantitation ("LLOQ").